Cobaltous acetate
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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-334

DATE: December 29, 1978

Thru: HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicology Branch, HFF-155

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigation of Cobaltous Acetate in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Cobaltous Acetate

Protocol:

Cobaltous Acetate (1) was tested for toxic and teratogenic effects in the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Cobaltous Acetate was toxic under all four conditions of test. The following LD_{50}s were obtained:

Air Cell:

0 Hours: 2.038 mg/kg (0.102 mg/egg)
96 Hours: 0.957 mg/kg (0.048 mg/egg)

Yolk:

0 Hours: 14.01 mg/kg (0.700 mg/egg)
96 Hours: 11.89 mg/kg (0.595 mg/egg)

Scattered abnormalities were observed under all conditions of test, but serious abnormalities were in no instance significantly higher than or different from those observed in the background. Cobaltous acetate displayed no teratogenicity under the test conditions employed.

1. Cobaltous Acetate, Harshaw Chemical Company Batch #452

2. McLaughlin, J., Marliac, J.P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol 5, 760-770

3. Verrett, M. J. Marliac, J.P., and McLaughlin, J., Jr., (1964) JOAC 47, 1002-1006

4. Finnery, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Cobaltous Acetate
Air Cell at 0 Hours

Table 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
2.50	50.00	115	100.00*	0.00	0.00
1.250	25.00	115	100.00*	1.73	0.00
0.50	10.00	115	99.13*	6.95	2.60
0.250	5.00	115	80.86*	4.34	0.86
0.1250	2.50	110	80.90*	6.36	0.00
Water Controls		125	34.40	1.60	1.60
		455	9.67	0.21	0.21

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 2.038 mg/kg (0.102 mg/egg)

Cobaltous Acetate
Air Cell at 96 Hours

Table 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
0.6250	12.50	110	100.00*	0.90	0.90
0.250	5.00	110	96.36*	1.81	0.90
0.1250	2.50	95	93.68*	6.31*	1.05
0.06250	1.25	95	70.52 *	4.21	0.00
0.0250	0.50	94	31.91	4.25	3.19
Water Controls		134	23.13	0.00	0.00
		455	9.67	0.21	0.21

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 0.9568 mg/kg (0.0478 mg/egg)

Cobaltous Acetate
Yolk at 0 Hours

Table 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	110	83.63*	1.81	0.00
2.50	50.00	110	76.36*	0.90	0.00
1.250	25.00	115	73.91*	3.47	3.47
0.50	10.00	115	63.47*	3.47	0.00
0.250	5.00	115	54.78*	0.00	0.00
Water Controls		124	32.25	0.80	0.80
		455	9.67	0.21	0.21

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 14.008 mg/kg (0.7004 mg/egg)

Cobaltous Acetate
Yolk at 96 Hours

Table 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
50.00	100.00	110	83.63*	2.72	0.00
2.50	50.00	109	75.22*	6.42	2.75
1.250	25.00	110	70.90*	12.72*	4.54
0.6250	12.50	109	57.79*	5.50	2.75
0.250	5.00	110	50.00*	4.54	0.90
Water		110	19.09	3.63	3.63
Controls		455	9.67	0.21	0.21

*Significantly different from solvent $p \leq 0.05$
**LD_{50} 11.890 mg/kg (0.595 mg/egg)